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## invited review

### Variable imprinting of the heterotrimeric G protein $G_s$ $\alpha$ -subunit within different segments of the nephron

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<sup>1</sup>Metabolic Diseases Branch, National Institute of Diabetes, Digestive, and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892; and <sup>2</sup>Division of Endocrinology and Metabolism, Department of Medicine, Georgetown University, Washington, District of Columbia 20007

Weinstein, Lee S., Shuhua Yu, and Carolyn A. Ecelbarger. Variable imprinting of the heterotrimeric G protein  $G_s$   $\alpha$ -subunit within different segments of the nephron. *Am J Physiol Renal Physiol* 278: F507–F514, 2000.—The heterotrimeric G protein  $G_s$  is required for hormone-stimulated intracellular cAMP generation because it couples hormone receptors to the enzyme adenylyl cyclase. Hormones that activate  $G_s$  in the kidney include parathyroid hormone, glucagon, calcitonin, and vasopressin. Recently, it has been demonstrated that the  $G_s\alpha$  gene is imprinted in a tissue-specific manner, leading to preferential expression of  $G_s\alpha$  from the maternal allele in some tissues. In the kidney,  $G_s\alpha$  is imprinted in the proximal tubule but not in more distal nephron segments, such as the thick ascending limb or collecting duct. This most likely explains why in both humans and mice heterozygous mutations in the maternal allele lead to parathyroid hormone resistance in the proximal tubule whereas mutations in the paternal allele do not. In contrast, heterozygous mutations have little effect on vasopressin action in the collecting ducts. In mice with heterozygous null  $G_s\alpha$  mutations (both those with mutations on the maternal or paternal allele), expression of the Na-K-2Cl cotransporter was decreased in the thick ascending limb, suggesting that its expression is regulated by cAMP. The  $G_s\alpha$  genes also generate alternative, oppositely imprinted transcripts encoding XL $\alpha$ s, a  $G_s\alpha$  isoform with a long NH<sub>2</sub>-terminal extension, and NESP55, a chromogranin-like neurosecretory protein. The role, if any, of these proteins in renal physiology is unknown.

genomic imprinting; Albright hereditary osteodystrophy; adenosine 3',5'-cyclic monophosphate; pseudohypoparathyroidism; parathyroid hormone

MANY HORMONES THAT REGULATE water and electrolyte transport in the kidney, such as parathyroid hormone (PTH), vasopressin, glucagon, and calcitonin, share a common signal transduction mechanism whereby receptor binding leads to increased intracellular concentrations of cAMP. The components necessary for hormone-stimulated cAMP generation are the receptor, the heterotrimeric G protein  $G_s$ , and adenylyl cyclase, which catalyzes the conversion of ATP to cAMP. The receptor is specific for each hormone whereas  $G_s$  and adenylyl cyclase are components common to all signaling pathways that lead to increased cAMP. The physiological responses to increased intracellular cAMP are cell-type specific. For example, PTH-stimulated cAMP generation in the proximal tubule leads to decreased

phosphate reabsorption and probably increased 1 $\alpha$ -hydroxylation of 25-hydroxyvitamin D whereas vasopressin-stimulated cAMP generation in the collecting ducts leads to increased water permeability.

Each heterotrimeric G protein is composed of an  $\alpha$ -,  $\beta$ -, and  $\gamma$ -subunit (for reviews, see Refs. 65, 70). The  $\beta$ - and  $\gamma$ -subunits form a tightly but noncovalently bound dimer. G proteins are identified by their specific  $\alpha$ -subunits, which bind guanine nucleotides and are important in both receptor and effector coupling. The  $\alpha$ -subunit of the G protein  $G_s$  is ubiquitously expressed and couples receptors to the stimulation of adenylyl cyclase and the opening of specific ion channels (53). There are at least nine isoforms of adenylyl cyclase. All bind to and are stimulated by  $G_s\alpha$  but differ in their regulation

by other intracellular compounds, such as  $\beta\gamma$ , calcium, and protein kinases A and C (64).

The genes encoding  $G_s\alpha$  are single copy in human (*GNAS1*) and mouse (*Gnas*). *GNAS1* is located at 20q13 (28, 29, 50) whereas *Gnas* is located in distal chromosome 2 within a region syntenic to human 20q13 (10, 59). It has recently been recognized that these genes are subject to genomic imprinting and generate multiple transcripts, some which are expressed only from the maternal allele and others that are expressed only from the paternal allele (31, 32, 60, 75). In mice (and probably in humans)  $G_s\alpha$  is expressed primarily from the maternal allele in some tissues but is biallelically expressed in other tissues (75).

Genomic imprinting is an epigenetic phenomenon affecting a small number of autosomal genes, which leads to preferential expression of either the maternal or paternal allele (for reviews, see Refs. 2 and 14). For example, the *Igf2* and *Snrpn* genes are only transcriptionally active on the paternal allele whereas the *Igf2r* and *H19* genes are only active on the maternal allele. Some genes (e.g., *Ube3a*, *Igf2*) are imprinted in a tissue-specific manner (1, 16), with a single allele active in some tissues and both alleles active in other tissues. Within imprinted genes are regions in which the maternal and paternal alleles are differentially methylated, which appear to be critical for both the initiation and maintenance of imprinting (14). Generally, the differential methylation patterns that maintain imprinting are erased in the primordial germ cell. Differential methylation which is reestablished in either the male or female gametes is presumed to represent the methylation imprint mark that distinguishes the maternal and paternal alleles in the offspring. Other differentially methylated regions are established later during postimplantation development. Genetic or epigenetic abnormalities involving imprinted genes have been implicated in several congenital disorders, such as the Angelman/Prader-Willi and Beckwith-Wiedeman syndromes, and in carcinogenesis. In this review we will summarize the evidence that *GNAS1* and *Gnas* are imprinted genes and that  $G_s\alpha$  is imprinted in a tissue-specific manner and discuss the physiological consequences of tissue-specific imprinting of  $G_s\alpha$  in the nephron.

### THE $G_s\alpha$ GENES ARE IMPRINTED

#### Albright Hereditary Osteodystrophy (AHO)

The first evidence for imprinting of *GNAS1* was provided by the unusual pattern of inheritance of AHO, a human disorder characterized by short stature, obesity, brachydactyly, subcutaneous ossifications, and mental defects and associated with null *GNAS1* mutations (70). AHO patients present with either the somatic features of AHO alone (termed pseudopseudohypoparathyroidism [PPHP]) or AHO plus resistance to multiple hormones that activate  $G_s$ -coupled pathways in their target organs (termed pseudohypoparathyroidism type Ia [PHP-Ia]). PHP-Ia patients, who are resistant to PTH, have a markedly reduced urinary cAMP

response to administered PTH (11) whereas in PPHP patients, who have normal serum calcium and PTH levels, the urinary cAMP response is normal (49). The defect was localized to  $G_s$  on the basis of the fact that  $G_s$  levels are decreased by ~50% in membranes isolated from various easily accessible cell types (e.g., erythrocytes, skin fibroblasts, platelets, and transformed lymphoblasts) (5, 20–22, 47, 48). In these tissues  $G_s\alpha$  expression is decreased by 50% (9, 45, 58) due to heterozygous-inactivating *GNAS1* mutations (57, 70, 72).

Both PHP-Ia and PPHP patients have similar decreases in  $G_s\alpha$  expression in accessible tissues (49) and identical *GNAS1* mutations (57, 70, 72). How identical *GNAS1* mutations lead to multihormone resistance in some patients (PHP-Ia) but not in others (PPHP) remained a mystery until it was noted that maternal transmission of AHO leads to offspring with PHP-Ia whereas paternal transmission leads to offspring with PPHP (15, 74), suggesting that *GNAS1* might be imprinted. If the *GNAS1* paternal allele is poorly expressed due to imprinting in a hormone target tissue (such as the proximal tubule, the major renal target for PTH), then a null mutation in the active maternal allele would markedly reduce  $G_s\alpha$  expression and lead to hormone resistance (PHP-Ia). In contrast, a null mutation in the relatively inactive paternal allele should have little effect on  $G_s\alpha$  expression and therefore little effect on hormone action (PPHP). This model is supported by the fact that the urinary cAMP response to exogenous PTH is markedly reduced in PHP-Ia patients but normal in PPHP patients (49). Although this model has been confirmed in mice (see below), it has yet to be definitively proven in humans, and in fact  $G_s\alpha$  has been shown to be expressed from both alleles in various human fetal tissues (7, 31, 32). This most likely reflects the fact that the imprinting is tissue specific, as there is no evidence for imprinting in various accessible tissues such as blood cells and fibroblasts, where  $G_s\alpha$  expression is equally decreased by 50% in both PHP-Ia and PPHP patients. Imprinting of  $G_s\alpha$  in humans can only be established by examining specific hormonal target tissues, such as the renal proximal tubule.

Further evidence for imprinting of  $G_s\alpha$  in humans is provided by a study that examined four kindreds affected with PHP type Ib (PHP-Ib) (39). PHP-Ib is characterized by PTH resistance in the absence of AHO or resistance to other hormones. Like PHP-Ia patients, PHP-Ib patients have a markedly reduced urinary cAMP response to PTH, localizing the defect to a signaling component including, or upstream of, adenyl cyclase (46, 63). Unlike PHP-Ia,  $G_s$  levels are normal in easily accessible tissues, ruling out a mutation involving the  $G_s\alpha$  coding region. Several lines of evidence rule out defects in the PTH receptor as the cause of PHP-Ib (4, 25, 26, 38, 61). In four PHP-Ib kindreds PTH resistance was only evident in individuals who inherited the trait from their mother (39), a pattern similar to the inheritance pattern of PTH resistance in PHP-Ia. Moreover, linkage analysis performed on these four kindreds mapped the disease gene

to 20q13, in the vicinity of *GNAS1*. One possible explanation for PTH resistance in these kindreds is an imprinting defect that impairs the switching of the paternal to maternal imprint in the female germline, leading to offspring with a paternal imprint in both alleles. This would drastically reduce  $G_s\alpha$  expression in the renal proximal tubules, leading to PTH resistance, but should not affect  $G_s\alpha$  expression in other tissues where  $G_s\alpha$  is equally expressed from the maternal and paternal alleles. Such imprinting defects have been identified in the *SNRPN* gene, leading to Prader-Willi and Angelman's syndromes (34).

#### The $G_s\alpha$ -Knockout (*GsKO*) Mouse Model

*Gnas* maps within a 20-centimorgan region on distal chromosome 2, which is presumed to have maternally and paternally imprinted genes because maternal and paternal uniparental disomies (UPDs) of the region result in distinct abnormal phenotypes (10). We generated mice with an insertion in the *Gnas* exon 2 coding sequence, resulting in a null *Gnas* allele (*GsKO*) (75). Homozygous *GsKO* mice ( $-/-$ ) die during early postimplantation development. Heterozygotes have distinct abnormal phenotypes determined by whether the maternal or paternal allele was disrupted.  $M-/+$  mice (those with a maternal *GsKO* allele) have wide, square-shaped bodies and subcutaneous edema at birth, and many develop neurological abnormalities and die 1–3 wk after birth. In contrast,  $+p-$  (those with a paternal *GsKO* allele) are born with narrow, arched backs, and most die within 1 day due to failure to suckle milk from their mother. The early phenotypes of  $m-/+$  and  $+p-$  mice are very similar to the phenotypes of mice with paternal UPD/maternal deletion and maternal UPD/paternal deletion of the imprinted region, respectively (10), strongly suggesting that the both the paternal and maternal phenotypes are caused by imprinting at the *Gnas* locus. In later development  $m-/+$  become obese whereas  $+p-$  mice have decreased lipid accumulation in adipose tissue relative to normal animals.

To directly determine the imprinting status of  $G_s\alpha$  in specific tissues, we measured the levels of  $G_s\alpha$  expression in  $m-/+$  and  $+p-$  mice relative to those in normal mice. In tissues where the paternal allele is poorly

expressed,  $G_s\alpha$  expression should be markedly reduced in  $m-/+$  mice and normal in  $+p-$  mice, whereas in tissues where  $G_s\alpha$  is not imprinted,  $G_s\alpha$  expression should be equally decreased by  $\sim 50\%$  in both  $m-/+$  and  $+p-$  mice.  $G_s\alpha$  mRNA and protein expression in renal proximal tubules is markedly reduced in  $m-/+$  mice and normal in  $+p-$  mice, consistent with imprinting of the *Gnas* paternal allele in this tissue. In contrast,  $G_s\alpha$  expression in the renal inner medulla (which consists primarily of collecting tubules) and outer medulla [which consists primarily of thick ascending limbs (TAL)] (43) was equally reduced by  $\sim 50\%$  in both  $m-/+$  and  $+p-$  mice (19, 75), consistent with lack of imprinting in these more distal portions of the nephron. In contrast to the results of Williamson and colleagues (73), we did not see a difference in *Gnas* expression between these two groups of mice in renal glomeruli, suggesting lack of imprinting in the glomerulus.  $G_s\alpha$  is also imprinted in some tissues outside of the kidney, such as brown and white adipose tissue, but is not imprinted in others, such as lung and skeletal muscle (Yu and Weinstein, unpublished observations). These results confirm that in mice  $G_s\alpha$  is imprinted in a tissue-specific manner and, specifically, that  $G_s\alpha$  is imprinted in the proximal tubules but not in other portions of the nephron.

#### Alternative *GNAS1* and *Gnas* Transcripts are Oppositely Imprinted

*GNAS1* was initially defined by the 13 exons that comprise the coding region for  $G_s\alpha$  (44). It is now clear that both *GNAS1* and *Gnas* are much more complex genes with multiple alternative promoters generating multiple alternative transcripts by splicing of alternative upstream exons to exon 2 (Fig. 1). One promoter located 35 kb upstream of the  $G_s\alpha$  promoter in *GNAS1* that generates transcripts encoding XL $\alpha$ s, a  $G_s\alpha$  isoform with a long  $NH_2$ -terminal extension, is active only on the paternal allele and methylated on the maternal allele (31, 40). In contrast, another promoter located 47 kb upstream of the  $G_s\alpha$  promoter that generates transcripts encoding NESP55, a chromogranin-like neurosecretory protein, is active only on the maternal allele and methylated on the paternal allele (32, 36). Exons

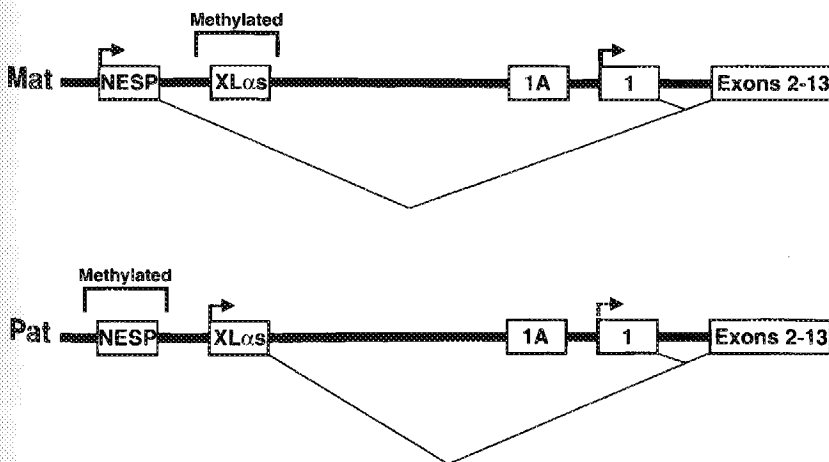


Fig. 1. Both *GNAS1* and *Gnas* have multiple oppositely imprinted transcripts. Schematic diagram showing maternal (Mat; top) and paternal (Pat; bottom) alleles of genes encoding  $G_s\alpha$ . Alternative upstream exons that splice into exon 2 to generate alternative transcripts encoding NESP55, XL $\alpha$ s, an unknown gene product, and  $G_s\alpha$ , are shown as boxes labeled NESP55, XL $\alpha$ s, 1A, and 1, respectively. Transcriptionally active promoters are designated by horizontal arrows, and regions of differential methylation are outlined above each allele. Dashed horizontal arrow for exon 1 in paternal allele indicates this promoter is active in some tissues and inactive in other tissues. No arrows are shown for exon 1A because imprinting status of this promoter has not been determined.

2-13 do not encode any portion of NESP55 but rather are found within the 3' untranslated region of the NESP55 mRNA. Both XL $\alpha$ s and NESP55 are expressed primarily in neuroendocrine tissues, and their biological functions have not been established. XL $\alpha$ s and NESP55 are also oppositely imprinted in mouse (Ref. 60; Yu and Weinstein, unpublished observations). The imprinting status of a fourth alternative promoter remains to be established (37, 67). *Gnas* is the only gene to date shown to have multiple oppositely imprinted transcripts in the sense direction.

If XL $\alpha$ s plays a major role in human physiology, then PPHP patients (in whom XL $\alpha$ s expression would be disrupted by mutations in the paternal allele) should have clinical manifestations that are not also found in PHP-Ia patients (in whom XL $\alpha$ s expression would be unaffected). In fact, this is not the case, suggesting that XL $\alpha$ s might not be critical in humans or that a closely related protein performs its function in PPHP patients. Similarly, several lines of evidence suggest that NESP55 might not be critical for human physiology or development. PHP-Ia patients with missense mutations within the  $G_{s\alpha}$  encoding region (54, 62, 68, 69) would likely have normal NESP55 expression because these mutations would not disrupt the NESP55 coding region or NESP55 mRNA expression. In contrast, early splice site and premature termination mutations would decrease NESP55 mRNA expression, similar to their effect on  $G_{s\alpha}$  mRNA expression (71, 72). In fact, there is little correlation between the type of mutation present and the clinical presentation of PHP-Ia patients. Also, the fact that exon 1 mutations (which should only disrupt  $G_{s\alpha}$  expression) and more downstream mutations (which should disrupt all *GNAS1* gene products) produce similar phenotypes suggests that most or all of the manifestations of AHO result from decreased  $G_{s\alpha}$  expression (57). Further studies are required to define the roles of XL $\alpha$ s and NESP55 in human physiology.

In contrast to humans, disruption of the maternal or paternal *Gnas* allele in mice leads to distinct pheno-

types with severe manifestations, including early lethality. The distinct phenotypes of  $m^{-/+}$  and  $+/p^{-}$  mice could be due to the disruption of paternally and maternally expressed transcripts resulting from our exon 2 insertion. The role for disruption of alternative *Gnas* transcripts in the  $m^{-/+}$  and  $+/p^{-}$  phenotypes is further supported by the observation that these developmental and lethal phenotypes are absent in mice with a *Gnas* exon 1 deletion, which should only disrupt the  $G_{s\alpha}$  transcript (W. F. Schwindinger and M. A. Levine, personal communication).

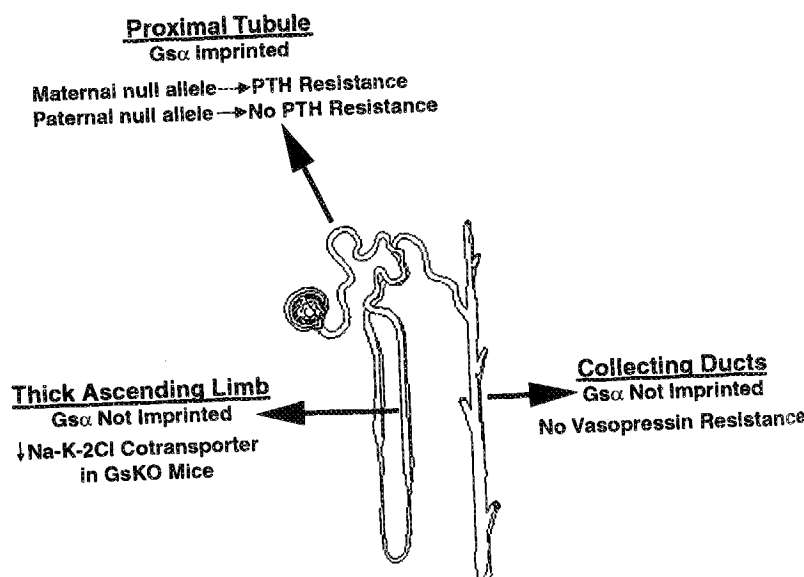
#### IMPLICATIONS OF $G_{s\alpha}$ IMPRINTING FOR RENAL PHYSIOLOGY

The imprinting status of  $G_{s\alpha}$  in the renal proximal tubule, TAL, and collecting ducts and the resulting physiological effects in AHO patients and GsKO mice are summarized in Fig. 2.

##### Renal Responses to PTH

PTH-stimulated cAMP generation in the proximal tubule leads to decreased phosphate reabsorption and increased synthesis of 1,25 dihydroxyvitamin D. In PHP-Ia patients, PTH-stimulated cAMP generation is markedly reduced, leading to decreased phosphate excretion and synthesis of 1,25 dihydroxyvitamin D. Decreased 1,25 dihydroxyvitamin D leads to hypocalcemia, because it is required for intestinal calcium absorption and calcium release from bone. These physiological abnormalities result in hypocalcemia, hyperphosphatemia, and elevated serum PTH, the clinical hallmarks of PTH resistance. In contrast, PTH-stimulated cAMP generation is normal in PPHP patients, and these patients show no evidence for PTH resistance. Until recently it was unclear how maternal inheritance of *GNAS1* mutations leads to PTH resistance (PHP-Ia) whereas paternal inheritance does not (PPHP). Similarly, we have recently shown in the GsKO mouse model that  $m^{-/+}$  mice have evidence for PTH resis-

Fig. 2. Imprinting status of  $G_{s\alpha}$  in different nephron segments and resulting physiological consequences in Albright hereditary osteodystrophy (AHO) patients and  $G_{s\alpha}$ -knockout (GsKO) mice.  $G_{s\alpha}$  is imprinted in proximal tubule with paternal allele poorly expressed, leading to parathyroid hormone (PTH) resistance when maternal allele is mutated [pseudohypoparathyroidism type Ia (PHP-Ia);  $m^{-/+}$  mice] but no PTH resistance when paternal allele is mutated (PPHP;  $+/p^{-}$  mice).  $G_{s\alpha}$  is not imprinted in the thick ascending limbs (TAL). Resulting ~50%  $G_{s\alpha}$  deficiency in both  $m^{-/+}$  and  $+/p^{-}$  mice leads to decreased expression of Na-K-2Cl cotransporter and decreased urinary concentrating ability in response to an acute exposure to vasopressin.  $G_{s\alpha}$  is not imprinted in collecting ducts, and heterozygous mutations are not associated with vasopressin resistance in inner medullary collecting ducts in either humans or mice.



tance (decreased serum ionized calcium, increased serum phosphorus and PTH, and decreased PTH-stimulated cAMP generation in proximal tubules) whereas  $+p-$  mice are not resistant to PTH (75). Furthermore, the differences in PTH-stimulated cAMP generation between  $m-/+$  and  $+p-$  mice correlate well with  $G_s\alpha$  expression in the proximal tubule, being markedly reduced in  $m-/+$  mice and normal in  $+p-$  mice. These findings confirm that the variable expression of PTH resistance in GsKO mice is due to imprinting of  $G_s\alpha$  in the proximal tubule and strongly suggest that the variable expression of AHO (PHP-Ia vs. PPHP) is also due to imprinting of  $G_s\alpha$ .

PTH also acts on more distal portions of the nephron to increase calcium reabsorption, and thus patients with primary hypoparathyroidism (deficient PTH secretion) are prone to hypercalciuria. In contrast, this action of PTH remains intact in PHP-Ia, and therefore it is uncommon for these patients to develop hypercalciuria (66). One possible explanation for this discrepancy is that  $G_s\alpha$  is not imprinted in the more distal portions of the nephron (e.g., TAL) where PTH affects calcium reabsorption. The predicted half-normal cAMP response to PTH in this portion of the nephron may be sufficient to produce a maximal physiological response, as has been shown for responses to glucagon and isoproterenol in PHP-Ia patients (6, 8, 46). It is also possible that the action of PTH to increase calcium reabsorption is maintained in PHP-Ia because this response is not dependent on cAMP (24).

#### Sodium Transport in the TAL

Both  $G_s\alpha$  expression and hormone-stimulated cAMP generation in the TAL are decreased by ~50% in both  $m-/+$  and  $+p-$  mice, and therefore  $G_s\alpha$  is not imprinted in the TAL in mice (19). Both acute (30, 33, 55) and chronic (3) exposure to vasopressin are associated with increases in chloride transport, and chronic exposure to a vasopressin analog or water restriction results in increased expression of the Na-K-2Cl cotransporter in the TAL (41). These observations and the presence of a cAMP-responsive element in the 5'-flanking region of the NKCC-2 gene (35), which encodes the Na-K-2Cl cotransporter, suggested that cAMP might have a positive effect on the expression of the cotransporter. If this is correct then expression should be decreased to a similar extent in both  $m-/+$  and  $+p-$  mice. The Na-K-2Cl cotransporter is decreased by ~50% in the outer medulla of both  $m-/+$  and  $+p-$  mice (19), consistent with the lack of imprinting of  $G_s\alpha$  in the TAL and providing further evidence for the regulation of the cotransporter by cAMP. Interestingly, the expression of adenylyl cyclase type 6 in the outer medulla was also decreased (19), suggesting a possible feed-forward regulatory mechanism by which decreased  $G_s\alpha$  levels could lead to decreased cAMP production. The Na-K-ATPase  $\beta_1$ -subunit was also decreased (19). In contrast, the expression of other apical TAL proteins, such as Tamm Horsfall protein and the type 3 Na-H exchanger, was unaffected, suggesting that the observed changes were specific and not due to generalized atrophy of the TAL.

A decrease in NaCl transport due to decreased Na-K-2Cl cotransporter abundance would be predicted to result in decreased countercurrent multiplication by the loop of Henle and decreased medullary solute concentrations. Consistent with this hypothesis, the urinary concentrating ability measured 1 h after intraperitoneal administration of vasopressin analog was significantly reduced by 28% in  $m-/+$  mice (19). One study in PHP-Ia patients found no significant decrease in urinary concentrating ability in response to acute intravenous vasopressin infusion, although only three patients were examined in this study (56). The decreased urinary concentrating ability observed in heterozygous GsKO mice could also be the result of vasopressin resistance in the collecting duct, although this possibility is less likely (see *Vasopressin Responses in the Collecting Duct*).

After water deprivation for 48 h, which leads to a maximal increase in circulating vasopressin levels, the abundance of the Na-K-2Cl cotransporter in the TAL was no longer different between mutant and wild-type animals (19). Consistent with this finding, no difference in maximal urinary concentrating ability could be detected after 48 h of water deprivation (19, 75). The most likely explanation for these observations is that chronic exposure to maximal vasopressin concentrations results in cAMP levels within the TAL of mutant mice that exceed those required for maximal stimulation of cotransporter expression.

#### Vasopressin Responses in the Collecting Duct

Both PHP-Ia patients (23, 56) and  $m-/+$  mice (19, 75) are able to concentrate their urine normally in response to water deprivation, consistent with a normal response to chronic vasopressin exposure. Vasopressin increases water permeability in the collecting duct chronically by increasing the expression of the aquaporin water channels AQP2 and AQP3 and acutely by translocating AQP2 to the apical membrane of the collecting duct principal cell (17, 18, 42, 52). Although AQP2 expression is decreased by ~50% in the cortical collecting ducts (CCD) of both  $m-/+$  and  $+p-$  mice, AQP2 expression in the inner and outer medulla is normal in these mice (Ref. 19; C. A. Ecelbarger and M. Knepper, unpublished observations). There are also no differences between mutant and normal mice in the expression of either adenylyl cyclase type 6 or the vasopressin-regulated urea transporter in the inner medulla. Normal expression of AQP2 in the inner medullary collecting ducts (IMCD) of GsKO mice might be due to either no effect on vasopressin-stimulated cAMP generation (because  $G_s$  is not rate limiting) or no effect of decreased cAMP levels on gene expression (because cAMP is still present at levels that do not limit AQP2 expression). It is also possible that adenylyl cyclase type 6 is rate limiting for cAMP generation (27), and therefore normal AQP2 expression reflects the fact that adenylyl cyclase type 6 is not decreased in the IMCD (as opposed to in the TAL).

It is interesting that, unlike in the IMCD, AQP2 expression is decreased in the CCD of both  $m-/+$  and

+/- mice. Decreased AQP2 expression might reflect lower basal levels of cAMP in the CCD because vasopressin-stimulated cAMP generation has been shown to vary between different portions of the collecting duct (51). After 48 h of water deprivation there were no longer differences in AQP2 expression in the CCD between mutant and normal mice (C. A. Ecelbarger and M. Knepper, unpublished observations), suggesting that, as in the TAL, maximal stimulation leads to cAMP levels that exceed the threshold required for a maximal downstream response (e.g., AQP2 expression). The similar effects in the collecting ducts of m-/+ and +/- mice are consistent with lack of  $G_{s\alpha}$  imprinting in the CCD and IMCD. It is unlikely that the observed decrease in AQP2 expression in the CCD leads to polyuria because the normal osmotic water permeability in the collecting ducts far exceeds that required to generate a maximally concentrated urine (13).

Whether there are defects in AQP2 translocation in response to vasopressin in GsKO mice has not been examined, although it is known that the levels of cAMP achieved by acute vasopressin administration greatly exceed those required for maximal osmotic water permeability (12). The lack of apparent vasopressin resistance in the collecting ducts of PHP-1a patients and GsKO mice probably reflects both the large excess of cAMP in the collecting ducts relative to the amount required for downstream cellular responses and the excess capacity for osmotic water permeability in the collecting ducts relative to that required to produce a fully concentrated urine.

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